

Amendments to the Specification

Page 2, please replace the paragraph beginning on line 1 (Paragraph [0004]) with the following:

Within chromosome 11q13 is a sixth centiMorgan interval that contains an estimated 120 genes. Of these genes, a large number code proteins that directly or indirectly affect bone metabolism, as shown in FIG. 1 and as described, for example by Levanon et al., Genomics 7: 65-74, 1990. FIG. 1 is a partial list of genes that have been mapped within this interval on chromosome 11q13. Genes in this list encode proteins with putative roles in bone metabolism. Some of these genes are known to co-localize specifically with OPS. Each gene that co-localizes is a known potential candidate for causing OPS and has been ~~labelled~~ labeled with an asterisk to denote this fact. Many of these genes are expressed in bone cells. For example, Chen et al. Genomics 55: 314-321 (1999) have studied a number of proteins that are expressed in bone cells and which seem to be coded by genes in this region.

Page 3, please replace the paragraph beginning on line 25 (Paragraph [0009]) with the following:

Another embodiment of the invention is an isolated DNA molecule useful for the diagnosis or therapy of osteoporosis having sequence homology to a region of the sequence shown in FIG. 2 that does not encode a polypeptide sequence but which is involved in regulation of bone ~~synthesis~~ synthesis. Yet another embodiment is a DNA molecule capable of specific hybridization under stringent conditions to a DNA molecule having sequence homology to a region of the sequence shown in FIG. 2 that does not encode a polypeptide sequence, and which, when introduced into human osteoblasts, increases the ability of the osteoblasts to synthesize bone. Yet another embodiment is a DNA molecule at least 15 nucleotides long that is complementary or homologous to a non-protein coding region of the sequence shown in FIG. 2, and which, when introduced into human osteoblasts, increases the ability of the osteoblasts to synthesize bone. In yet another embodiment, the introduced complementary or homologous DNA molecule is at least 25 nucleotides long and in yet another embodiment the DNA molecule is at least 50 nucleotides long. In yet another embodiment, the isolated DNA or RNA molecule that is complementary or homologous to the non-protein coding region of the sequence shown in FIG. 2 is used as a predictor of osteoporosis. In another embodiment the DNA or RNA is used in

a binding reaction with DNA or RNA obtained directly or indirectly from a patient sample to to determine propensity of the patient to osteoporosis.

Page 6, please replace the paragraph beginning on line (Paragraph [0027]) with the following:

The inventors have discovered that a mutation in a gene previously known as LRP5, Lrp7, or Lr3 causes OPS and generally affects the regulation of bone strength and mineralization through various molecular interactions. A second discovery is that amino acid alterations at particular locations in that gene, herein termed the "bone strength and mineralization regulator" ("BSMR") gene lead to genetic polymorphisms that account for a significant portion of normal variability in bone strength and mineralization among individuals. A third discovery is that the BSMR gene modulates biochemical functions leading to alterations in bone strength and density according to its copy number, affinity of binding sites on the BSMR protein (extracellular region adjacent to the ~~membrane~~ membrane surface) to ligand and other biochemical events such as modulating angiogenesis.

Page 7, please replace the paragraph beginning on line 12 (Paragraph [0029]) with the following:

These discoveries have led to specific embodiments of the invention that are useful for the diagnosis and treatment (both ~~prophylatic~~ prophylactic and therapeutic) of osteoporosis. The inventors furthermore have discovered that the BSMR gene system regulates other biological systems such as blood vessel growth in the eye. Although the present disclosure emphasizes diagnosis and treatment of osteoporosis through BSMR regulation of bone cells, other analogous regulatory systems that utilize the BSMR gene are detected and altered in a like manner to that described for bone.

Page 9, please replace the paragraph beginning on line 5 (Paragraph [0035]) with the following:

Alkaline phosphatase activity is exemplified as a preferred metabolic reaction in many embodiments. However, a skilled artisan is also familiar with a number of other chemical and/or physical reactions that indicate bone anabolism and which can be used. One preferred technique is to monitor the binding of BSMR with another effector by using ~~fluoresence~~ fluorescence resonance energy transfer between a fluor on BSMR and a fluor on the effector as the two fluors become close through binding of effector to BSMR. This technique works well to monitor binding of BSMR with axin and other components of the BSMR regulatory system as well. Accordingly one embodiment is to use this technique in pluripotent cell lines that can go down

the osteoblastic lineage or even in osteoblastic cell lines to find extracellular ligands (BSMR effectors) for BSMR signaling.

Page 9, please replace the paragraph beginning on line 28 (Paragraph [0038]) with the following:

An embodiment of the invention provides a ligand discovery system that identifies pharmaceutically active substances that can bind BSMR and affect bone strength and mineralization. The inventors have discovered that upregulating this bone strength and mineralization system can improve bone status not only in individuals having a defective gene, but also in normal individuals that may desire increased bone strength or mass. This embodiment of the invention is carried out by one or more of: (a) adding an exogenous pharmaceutical that binds to BSMR; (b) altering the concentration of a naturally ~~occurring~~ occurring ligand that binds to BSMR; (c) activating a "pathway gene" that encodes a product that is associated with the BSMR protein regulatory pathway; (d) transgenically adding the BSMR gene or a pathway gene to desired cells; (e) activation of a BSMR gene in bone cells; (f) inactivating a pathway gene that encodes a product that is associated with the BSMR protein regulatory pathway and (g) adding an exogenous pharmaceutical that affects the BSMR protein regulatory pathway.

Page 10, please replace the paragraph beginning on line 18 (Paragraph [0040]) with the following:

In other embodiments biological functions that are controlled by the BSMR gene similarly may be regulated by any of (a) to (e). In yet other embodiments, the BSMR gene, or a portion that codes for the ligand binding portion of the BSMR protein is combined with another characteristic sequence that is useful to monitor the protein or to immobilize it. Example 2 discloses use of a FLAG FLAG[®] antibody epitope in this context, and Example 3 demonstrates insertion of a human IgG1 Fc domain, which can detect BSMR polypeptide, as well as having the BSMR engage in binding reactions (or immobilization to a solid surface) based on reactivity of the added Fc domain with an Fc binding partner, as is known in the art.

Page 10, please replace the paragraph beginning on line 26 (Paragraph [0041]) with the following:

In another embodiment a portion of the BSMR protein containing one or more ligand binding regions is used. The examples demonstrate the use of a BSMRdelta TM decoy construct.

The construct has carboxyl-terminal myc and HIS tagged epitopes that are fused in frame with the BSMR extracellular domain, which contains the ligand binding sites. This fusion protein was found to be readily secreted from cells and functional as an inhibitor of Wnt signaling in vitro. Still further, the fusion protein was found to inhibit bone accrual in calvarial explant cultures. This protein and others like it that a skilled artisan may derive based on this disclosure is particularly useful for drug design, discovery of related biochemistry relating to the Wnt system and as a therapeutic itself. Such a fusion protein can be purified, for example, using a nickel column or an ~~anti-mycantibody~~ anti-myc antibody column.

Page 11, please replace the paragraph beginning on line 27 (Paragraph [0045]) with the following:

FIG. 4 shows some BSMR coding sequence variations that have been found by the inventors. The term "Source" in this figure refers to the genomic DNA source. The term "OPS" indicates that the respective DNA came from affected OPS patients. The term "Control" indicates that the DNA studied was anonymous DNA samples from a healthy individual. The inventors have discovered the third listed alteration of a leucine insertion into residue 12, within a leucine stretch within the signal peptide region of the protein at a frequency of approximately 20 of 100 control individuals. This yields an estimated allele frequency of 0.1. One embodiment of the invention is a diagnostic tool for detecting the addition of one, two, three, four, five or more leucines to this stretch of leucines, as addition of one or more leucines here has an effect on the protein's functioning. The detection of this polymorphism and related leucine addition polymorphisms within this particular polyleucine spot is ~~proenestie~~ prognostic for osteoporosis tendency and normal bone density tendency. Detection of such leucine insertion polymorphisms specifically is contemplated for embodiments of the invention. In one embodiment, multiple leucines are added to the region.

Page 18, please replace the paragraph beginning on line 6 (Paragraph [0077]) with the following:

In one embodiment a regulatory protein useful for practice of the invention has a protein sequence described in ~~FIG. No. 2~~ FIG. 2 or a variant of such sequence. Through the manipulation of a nucleotide sequence by standard molecular biology techniques, variants of the BSMR protein may be made which differ in precise amino acid sequence from the disclosed

proteins yet which maintain the essential characteristics of the BSMR protein or which may be selected to differ in some characteristic way. Such variants are another embodiment of the invention.

Page 19, please replace the paragraph beginning on line 19 (Paragraph [0082] with the following:

Of course, peptides related to the above described proteins will have activity in influencing the BSMR regulatory system and are ~~desireable~~ desirable for embodiments of the invention. The peptides shown in FIG. 13 in this context are desirable and generally may stimulate the BSMR system, leading to increased bone metabolism, as could be seen by measuring alkaline phosphatase activity or another metabolic reaction affecting bone mineralization.

Page 22, please replace the paragraph beginning on line 30 (Paragraph [0086]) with the following:

In many embodiments extracellular ligands are particularly desirable to modulate bone strength and mineralization. The ligands operate by binding to one or more extracellular ligand binding repeats of the regulatory protein and thereby activate biochemical and cellular events that lead to increased bone strength and mineralization. In one embodiment, the ligand achieves this effect by causing a cell which expresses the regulatory protein on its surface, to proliferate and/or differentiate into an osteoblast cell. In another embodiment the ligand achieves this effect by inhibiting a cell which expresses the regulatory protein on its surface from proliferating or from differentiating into an osteoclast cell. In yet another embodiment the ligand ~~achieves this effect by~~ achieves this effect by removing a catabolic extracellular matrix factor such as an enzyme or growth receptor by binding to the matrix factor and preventing the factor from interacting with other substances.

Page 22, please replace the paragraph beginning on line 1 (Paragraph [0091]) with the following:

Example 11 (See Fig. 11) demonstrates the effect of BSMR overexpression. FIGS. 11a to 11c show the effect of inducing alkaline phosphatase activity in pluripotent cells that enter the

{PAD2232.DOC;2}

osteoblastic lineage, and the effect of Wnt3 on overexpressed BSMR. FIG. 11a shows enhancement of Wnt3a-mediated TCF--1 activation by ~~overexpression~~ overexpression of BSMR in COS cells. Accordingly, in embodiments of the invention, BSMR is increased, causing the effects demonstrated by these working examples. Methods of increasing expression, for example by transgenic expression of foreign genes, gene regulators and the like are known and new methods will be developed in the future for upregulating BSMR, now that the link between the BSMR gene and bone mineralization is established.

Page 22, please replace the paragraph beginning on line 30 (Paragraph [0094]) with the following:

In another desirable embodiment an extracellular antagonist alters bone strength and mineralization by interfering with binding of a stimulatory ligand to the BSMR protein. Some examples of stimulatory ligands are given in FIG. 13. For example, dkk--1, dkk--2, dkk--3, RAP, sFRP and human analogues of these proteins can interfere and have various degrees of activity. In a preferred embodiment a polynucleic acid encoding a gene for a naturally ~~occurring~~ occurring antagonist of BSMR activity is added in an antisense format such that an antisense strand forms and binds to messenger RNA, thus blocking translation and/or triggering nuclease destruction of the RNA. Recombination using antisense technology has previously been used to inhibit expression of specific gene products in mammalian cell lines (Kasid et al., Science 243:1354-1356 1989; Khoka et al., Science 243:947-950 1989; Izant et al., Science 229:345-352 1985) including some retroviruses; (von Ruden et al., J. Virol. 63:677-682 1989; and Chang et al., J. Virol. 61:921-924 1987). Each of these methods, and later developed methods specifically are contemplated for this embodiment of the invention.

Page 27, please replace the paragraph beginning on line 1 (Paragraph [0107]) with the following:

One type of controlled release system utilizes mechanical attachment of polymer to bone. Briefly, a biodegradable polyester film, such as made from polylactic acid, polyglycolic acid, polydioxanone or polylactic acid/polyglycolic acid copolymer film, containing the effector is prepared and fabricated into a pin, plate, screw or the like for attachment to or insertion into bone. The compositions provide sustained release of the effector at the target site. Fifty percent PLA and fifty percent PGA films are preferred. These films further may include a carrier such as

collagen, a recombinant collagen, ~~Ossigel™~~ collagen sold under the trademark OSSIGEL™ from Orquest Inc., Healos mineralized collagen matrix from Orquest Inc., albumin and/or a detergent such as polyoxyethylenesorbitan. When a protein such as albumin is included, the ratio of effector to the protein preferably in general is maintained between 0.01 and 10 milligrams effector per gram of protein. In principle, any substance that enhances polymer degradation, creates pores in the film or reduces adsorption of the effector(s) to the film can be used as a carrier. Human albumin is a particularly preferred carrier.

Page 28, please replace the paragraph beginning on line 7 (Paragraph [0111]) with the following:

Alternative methods for local delivery of BSMR effector and/or an additional agent useful for bone growth, regulation or repair include use of ~~ALZET~~ ALZET™ osmotic minipumps (Alza Corp. Palo Alto, Calif.); sustained release matrix materials such as those disclosed in Wang et al. (WO 90/11366); electrically charged dextran beads as disclosed in Bao et al. (WO 92/03125); collagen-based delivery systems, for example, as disclosed in Ksander et al. (Ann. Surg. 211(3):288-294, 1990); methylcellulose gel systems as disclosed in Beck et al. (J. Bone Min. Res. 6(11):1257-1265, 1991) and alginate-based systems as disclosed in Edelman et al. (Biomaterials, 12:619-626, 1991). Other methods well known in the art for sustained local delivery in bone include porous coated metal ~~protheses~~ prosthesis that can be impregnated and solid plastic rods with therapeutic compositions incorporated within them.

Page 28, please replace the paragraph beginning on line 10 (Paragraph [0114]) with the following:

In another embodiment of the invention, test substances such as peptides and synthetic chemicals are screened for their ability to interact with one or more components of the BSMR system. The screen may test for binding to BSMR directly, such as to an extracellular site on the protein, to an intracellular site on the protein, or to another protein that interacts with BSMR. Typically in a screen a test substance is incubated with a specific target of the BSMR system and binding is detected. The term "binding" in this context means that the association (determined by measuring an ~~asociation~~ association constant) between test substance and the BSMR component is greater than the association between the same test substance and other protein generally. Preferably the binding between test substance and BSMR component is at least 10 times as

strong and more preferably is at least 100 times as strong. Human serum albumin is preferred as a reference "other protein" because a large proportion of extracellular fluid protein is serum albumin.

Page 29, please replace the paragraph beginning on line 10 (Paragraph [0115]) with the following:

In one embodiment a useful compound for screening preferably is a natural product, peptide, or other small compound capable of mimicking the effects of a naturally ~~occurring~~ occurring regulator such as a Wnt protein. One preferred screening method uses the ~~β-catenin/TCF signalling~~ β-catenin/TCF signaling, another uses G--protein based biochemical reactions, and another uses downstream ~~signalling~~ signaling pathway molecules based on the above identified reactive binding partners. The screening methods also may utilize BSMR target genes specific to osteoblasts or use the BSMR natural promoter to identify BSMR inducers.

Page 30, please replace the paragraph beginning on line 10 (Paragraph [0119]) with the following:

In an embodiment, a compound for screening may be a natural product, peptide, or other small compound capable of mimicking the effects of a naturally ~~occurring~~ occurring regulator such as a Wnt protein. One preferred screening method uses the ~~β-catenin/TCF signalling~~ β-catenin/TCF signaling, another uses G--protein based biochemical reactions, and another uses downstream ~~signalling~~ signaling pathway molecules based on the above identified reactive binding partners. The screening methods also may utilize BSMR target genes specific to osteoblasts or use the BSMR natural promoter to identify BSMR inducers.

Page 33, please replace the paragraph beginning on line 1 (Paragraph [0128]) with the following:

In another desirable embodiment an extracellular antagonist alters bone strength and mineralization by interfering with binding of a stimulatory ligand such as one of the stimulatory ligands shown in FIG. 13, to the BSMR protein. For example, dkk--1, dkk--2, dkk--3, RAP, sFRP and human analogues of these proteins can interfere and have various degrees of activity. In a preferred embodiment a polynucleic acid encoding a gene for a naturally ~~occurring~~ occurring

antagonist of BSMR activity is added in an antisense format such that an antisense strand forms and binds to messenger RNA, thus blocking translation and/or triggering nuclease destruction of the RNA. Recombination using antisense technology has previously been used to inhibit expression of specific gene products in mammalian cell lines (Kasid et al., Science 243:1354-1356 1989; Khoka et al., Science 243:947-950 1989; Izant et al., Science 229:345-352 1985) including some retroviruses; (von Ruden et al., J. Virol. 63:677-682 1989; and Chang et al., J. Virol. 61:921-924 1987). Each of these methods, and later developed methods specifically are contemplated for this embodiment of the invention.

Page 39, please replace the paragraph beginning on line 10 (Paragraph [0147]) with the following:

Oligonucleotides specific to normal or mutant sequences are chemically synthesized using commercially available machines, ~~labelled~~ labeled radioactively with isotopes (such as ³²P) or non-radioactively (with tags such as biotin (Ward and Langer et al. (1981). Proc. Natl. Acad. Sci. USA 78:6633-6657), and hybridized to individual DNA samples immobilized on membranes or other solid supports by dot-blot or transfer from gels after electrophoresis. The presence or absence of these specific sequences are determined optically by methods such as autoradiography or fluorometric (Landegren, et al., 1989) or calorimetric reactions (Gebeyehu et al. (1987). Nucleic Acids Res. 15:4513-4534).

Page 41, please replace the paragraph beginning on line 8 (Paragraph [0156]) with a the following:

With the provision of the BSMR cDNA and the understanding of the role and mechanisms of the coded protein in bone strength and mineralization, vectors that contain BSMR DNA may be used for diagnosis and therapy. Furthermore, the DNA sequence of the BSMR cDNA and polymorphic or mutated BSMR cDNAs isolated from OPS patients and other patients can be manipulated in studies to further understand the expression of the gene and the function of its product. In this way, further ligands ~~usful~~ useful for embodiments of the invention and mechanisms involved in control of mineralization can be discovered.

Page 45, please replace the paragraph beginning on line 22 (Paragraph [0169] with the following:

The ligated product is transfected into mouse NIH 3T3 cells using ~~Lipofectin~~ a liposome preparation formulated from cationic lipids sold under the trademark LIPOFECTIN® (Life Technologies, Inc.) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in ~~600 ng/ml~~ 600 µg/ml G418 (Sigma, St. Louis, Mo.). The protein is released into the supernatant and may be purified by standard immunoaffinity chromatography techniques using antibodies raised against the BSMR protein, as described below.

Page 45, please replace the paragraph beginning on line 28 (Paragraph [0170] with the following:

Expression of the BSMR protein in eukaryotic cells may also be used as a source of proteins to raise antibodies. The BSMR protein may be extracted following release of the protein into the supernatant as described above, or, the cDNA sequence may be incorporated into a eukaryotic expression vector and expressed as a chimeric protein with, for example, ~~beta-globin~~ β-globin. Antibody to ~~beta-globin~~ β-globin is thereafter used to purify the chimeric protein. Corresponding protease cleavage sites engineered between the ~~beta-globin~~ β-globin gene and the cDNA are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating ~~beta-globin~~ β-globin chimeric proteins is pSG5 (Stratagene). This vector encodes rabbit ~~beta-globin~~ β-globin.

Page 46, please replace the paragraph beginning on line 18 (Paragraph [0172] with the following:

Retroviruses have been considered the preferred vector for experiments in gene therapy, with a high efficiency of infection and stable integration and expression (Orkin et al. (1988). Prog. Med. Genet. 7:130). The full length BSMR gene or DNA can be cloned into a retroviral vector and driven from either its endogenous promoter or from the ~~retroviral~~ retroviral LTR (long terminal repeat). Other viral transfection systems may also be utilized for this type of approach, including Adeno-Associated virus (AAV) (McLaughlin et al. (1988). J. Virol. 62:1963), Vaccinia virus (Moss et al. (1987). Annu. Rev. Immunol. 5:305), Bovine Papilloma virus

(Rasmussen et al. (1987). Methods Enzymol. 139:642) or members of the herpesvirus group such as Epstein-Barr virus (Margolskee et al. (1988). Mol. Cell. Biol. 8:2837-2847).

Page 48, please replace the paragraph beginning on line 10 (Paragraph [0180] with the following:

Another embodiment of the invention is an immunoassay for detecting predisposition for heavy bone mass, comprising antibodies that specifically bind BSMR protein, wherein the antibodies are selected from the group consisting of monoclonal antibodies and polyclonal antibodies. Techniques for using the antibodies in a kit (that is, in a package with an instructional chart or package insert) are ~~routine~~ routine and are contemplated as further embodiments of the invention.

Page 56, please replace the paragraph beginning on line 19 (Paragraph [0201] with the following:

Oligonucleotides specific to normal or mutant sequences are chemically synthesized using commercially available machines, ~~labelled~~ labeled radioactively with isotopes (such as ~~sup-32 P~~ ³²P) or non-radioactively (with tags such as biotin (Ward and Langer et al. Proc. Natl. Acad. Sci. USA 78:6633-6657 1981), and hybridized to individual DNA samples immobilized on membranes or other solid supports by dot-blot or transfer from gels after electrophoresis. The presence or absence of these specific sequences are visualized by methods such as autoradiography or fluorometric or colorimetric reactions (Gebeyehu et al. Nucleic Acids Res. 15:4513-4534 1987).

Page 61, please replace the paragraph beginning on line 29 (Paragraph [0221] with the following:

Using standard techniques, a full length wild type construct extending from primers LRCOD3F to LRCOD12R was constructed. A sequence encoding a FLAG antibody epitope (GAC TAC AAG GAC GAC GAT GAC AAG) (SEQ ID NO:84) was inserted into the wild-type construct immediately downstream of nucleotide 165 (relative to the "A" in the ATG translation start site). This construct expressed a BSMR protein having a FLAG FLAG[®] epitope between wild type BSMR amino acid residues 55 and 56. The construct was prepared by the following

primer sequences and a ~~Quickchange reaction~~ using a site-directed mutagenesis reaction sold under the trademark QUICKCHANGE®:

Page 62, please replace the paragraph beginning on line 11 (Paragraph [0222] with the following:

Using standard techniques, a construct was made by inserting a sequence encoding an MYC antibody epitope (GAG CAG AAG CTG ATA TCC GAG GAG GAC CTG) (SEQ ID NO:87) immediately ~~upstream~~ upstream of the stop codon after residue 4845 (relative to the "A" in the ATG translation start site). The construct expressed a BSMR protein having a MYC epitope at the end of the wild type BSMR polypeptide. This construct was constructed using the following primer sequences and a ~~Quickchange reaction~~ using a site-directed mutagenesis reaction sold under the trademark QUICKCHANGE®:

Page 62, please replace the paragraph beginning on line 25 (Paragraph [0223] with the following:

Using standard techniques, an ~~expression~~ expression construct containing both the ~~FLAG~~ FLAG® and MYC antibody epitopes as described above at the aforementioned sites was produced.

Page 63, please replace the paragraph beginning on line 1 (Paragraph [0225] with the following:

An in-frame Hind III restriction enzyme site was created in a first full length wild-type BSMR construct, at the ~~amino-terminus~~ amino-terminus of the transmembrane domain, at nucleotide residue 4248. Separately, an in-frame Hind III restriction enzyme site was created in a second full length wild-type BSMR construct at the carboxy terminus. Using the Hind III restriction sites in the first construct, a Hind III/Not I fragment of the human IgG1 Fc domain (GenBank accession X70421.1) was inserted in-frame to create a functional Fc epitope at the carboxy-end of the BSMR extracellular domain.

Page 63, please replace the paragraph beginning on line 22 (Paragraph [0228]) with the following:

In this example conditioned media containing the dominant-negative secreted form of BSMR reduced alkaline phosphatase expression in primary calvarial osteoblast cultures and reduced bone formation in calvarial explant cultures. Calvarial cells exposed to Wnt3a conditioned media increased their alkaline phosphatase activity compared to controls. Cells exposed to conditioned media from cells expressing wild type BSMR did not differ from controls. Cells exposed to conditioned media from cells expressing the secreted BSMRdeltaTM form of BSMR had reduced alkaline phosphatase activity compared to controls, indicating that the decoy BSMR receptor had interfered with osteoblast function. In another study, explants that were cultured in the presence of conditioned media from cells expressing BSMRdeltaTM consistently had less bone formation, as ~~determined~~ determined by von Kossa staining, than did explants cultured in conditioned media from cells expressing wild-type BSMR. This BSMR is able to bind extracellular growth factors, such as Wnts, that are important during bone growth.

Page 66, please replace the paragraph beginning on line 20 (Paragraph [0240]) with the following:

BSMR expression constructs were constructed using the pcDNA3 expression vector (Invitrogen). Full length cDNA was amplified by RT-PCR from human fibroblasts and subcloned. A FLAG FLAG[®] antibody epitope (~~GAC TAC AAG GAC GAC GAT GAC AAG~~) 5'-GAC TAC AAG GAC GAC GAT GAC AAG-3' (SEQ ID NO:84) was inserted into the wild-type construct immediately downstream of nucleotide 165 (the insertion site is relative to the "A" in the ATG translation start site) using ~~QUIKChange~~ a site directed mutagenesis kit sold under the trademark QUICKCHANGE[®] (Stratagene). ~~QUIKChange~~ QUICKCHANGE[®] was also used to create expression constructs containing the 1270fs1438stop and Gln853Stop disease-causing mutations. An endogenous Pml I restriction site, downstream of the transmembrane domain, was used to generate the BSMRdeltaC dominant negative receptor by truncating the protein and removing the intra-cytoplasmic tail. An endogenous Apa I restriction site at the beginning of the transmembrane domain was used to generate the BSMRdeltaTM dominant negative receptor that lacks the transmembrane domain and cytoplasmic tail. The entire BSMR coding sequences of all constructs were confirmed by sequencing. Stable ST2 cell lines expressing either wild type or a second LRP5□C' construct, which was generated using an endogenous BsrG I restriction

enzyme site and lacks the final 100 amino residues of the cytoplasmic tail, were created picking individual colonies following selection with G418.

Page 67, please replace the paragraph beginning on line 7 (Paragraph [0242]) with the following:

Mouse TCF--1 was amplified by RT--PCR and the nucleotide sequence was confirmed by DNA sequence analysis and cloned into pcDNA3.1 vector (Invitrogen). The TCF--luciferase reporter construct, ~~TOPflash~~, sold under the trademark TOPFLASH™, was obtained from Upstate Biotechnology.

Page 67, please replace the paragraph beginning on line 12 (Paragraph [0244]) with the following:

Cell lines were cultured (5% CO₂ at 37° C.) respectively in \square --MEM, RPMI and DMEM medium supplemented with 10% of heat inactivated fetal calf serum. ST--2 stable cell lines were maintained in the corresponding culture medium and supplemented with G418 (500 μ g/ml). For treatment or transient transfection, cells were plated at 2X10⁴/cm² and 24 h later treatment or transfections were carried out as indicated below. Cells plated in 24-well plates were transiently transfected with the indicated construct (1 microgram) using ~~DNA-lipid complex Fugene-6~~ a DNA-lipid complex sold under the trademark FUGENE 6™ (Boehringer ~~Maheim~~ Manheim) according to the manufacture's protocol. For luciferase reporter assays 20 ng of pRL-TK (Promega, Madison, WI), which encodes a Renilla luciferase gene downstream of a minimal HSV-TK promoter, was systematically added to the transfection mix to assess transfection ~~effieienty~~ efficiency. When required, controls were carried out by replacing constructs with corresponding empty vectors.

Page 67, please replace the paragraph beginning on line 22 (Paragraph [0245]) with the following:

Sixteen hours after transfection, cells were washed, cultured in medium at 2% fetal calf serum and either left unstimulated or stimulated with BMP-2 (100 ng/ml) for additional 48 h. Luciferase or alkaline phosphatase activity was determined in cell lysates. When luciferase reporter constructs were used, luciferase assays were performed with ~~the Dual Luciferase Assay Kit~~ a kit sold under the trademark DUAL LUCIFERASE ASSAY KIT™ (Promega) according to

the manufacturer's instructions. Ten μ l of cell lysate was assayed first for firefly luciferase and then for Renilla luciferase activity. Firefly luciferase activity was normalized to Renilla luciferase activity. Alkaline phosphatase activity was determined in cell lysates using ~~the Alkaline Phosphatase Opt kit~~ a kit sold under the trademark ALKALINE PHOSPHATASE OPT KIT™ (Roche Molecular Biochemicals). Cell lysates were analyzed for protein content using ~~the micro-BCA Assay kit~~ a kit sold under the trademark MICRO-BCA ASSAY KIT™ (Pierce), and alkaline phosphatase activity was normalized for total protein concentration. Each experiment was performed in triplicate, and was repeated three times. Data (plus and minus one standard deviation) from one representative experiment are presented in FIG. 11.

Page 68, please replace the paragraph beginning on line 10 (Paragraph [0248]) with the following:

BSMR conditioned media was generated by transiently transfecting 80% confluent COS-7 cells in 100 mm diameter dishes with 5 μ g plasmid DNA using ~~Fugene-6~~ a DNA-lipid complex sold under the trademark FUGENE 6™ following the manufacturer's protocol. 10 ml each of conditioned medium was collected 24 and 48 hours after the cells were allowed to recover from the transfection. The presence of the protein in either the conditioned media or cell extract was confirmed by Western blotting with the ~~anti-flag~~ anti-FLAG® antibody.

Page 69, please replace the paragraph beginning on line 21 (Paragraph [0250]) with the following:

Murine calvaria cells were obtained from neonatal mice 1-2 days after birth by sequential collagenase digestion. Calvariae were removed and incubated at 37° C. in DMEM containing trypsin (0.5 mg/ml) and EDTA (1.5 mg/ml) under continuous agitation. Trypsin digests were discarded at 15 minutes and replaced with DMEM that contained 1 mg/ml of collagenase. ~~eThe~~ The collagenase digests were discarded at 20 minutes and replaced with fresh enzyme dilution. The cells released between 20-40 minutes were collected by a short passive sedimentation step, and two centrifugation steps (400g, 10 minutes), The collected cells were cultured in proliferation medium (DMEM supplemented with 20% FCS and 2 mM glutamine) at a density of 2.5X10⁴ cells per cm² in Petri dishes (100 mm diameter). Calvaria cells were cultured until 80% confluence (time 0) and proliferation medium was replaced by differentiation medium (DMEM

containing 10% FCS, 2 mM glutamine, 50 mg/ml ascorbic acid and 10 mM b--glycerolphosphate). Total RNAs were extracted with an ~~RNAplus~~ RNAPLUS™ kit from Quantum, from cells harvested from culture at days 0, 2, 7, 14, and 21. Changes in relative gene expression were assessed by using GeneChips™ DNA microarray slides sold under the trademark GeneChips® (Affymetrix). Results (see FIG. 7) are expressed in ratios using the time 0 as denominator, significant changes in expression ($pval < 0.1$ and ratios $> 1.5X$) are indicated with **.

Page 69, please replace the paragraph beginning on line 21 (Paragraph [0253]) with the following:

Both cell populations were harvested and dual ~~labelled~~ labeled for STRO--1 and alkaline phosphatase as described by Stewart, K et al, JBMR 11:P 208 (1996) prior to sorting by flow cytometry. Sort regions were the set within each of the quadrants and cells were sorted into four populations. Cells recovered were re-analyzed by flow cytometry for purity, counted, then pelleted and stored at -80° C. The STRO--1+ fraction corresponded to less differentiated osteoblast ~~predecessors~~ precursors (R5), the STRO--1+/AP+ fraction corresponded to more mature osteoblasts (R3) and the AP+ fraction corresponded to mature osteoblasts (R2). Total RNAs were extracted with a ~~RNAplus~~ an RNAPLUS™ kit provided by Quantum. Changes in relative gene expression were assessed by GeneChips™ DNA microarray slides sold under the trademark GeneChips® from Affymetrix. Results (see FIG. 8) are expressed in ratios with values at time 0 as denominators. Significant changes in expression ($pval < 0.1$ and ratios $> 1.5X$) are indicated with **. Ratios greater than 1.5 and with $pval < 0.15$ are indicated with *.

Page 70, please replace the paragraph beginning on line 3 (Paragraph [0254]) with the following:

This example shows transcriptional regulation of frizzled receptors (1 and 4) and SFRP2 observed in mouse pluripotent and osteoblast-like cell lines. The cells described as above were treated as indicated with BMP2, BMP/SHH or TGFbeta and changes in relative gene expression were assessed using GeneChips™ DNA microarray slides sold under the trademark GeneChips® by Affymetrix. Results (see FIG. 9) are expressed in ratios using the expression values of the untreated cells in the denominator. Only the significant changes in expression ($pval < 0.1$ and ratios $> 1.5 X$) are shown.

Page 71, please replace the paragraph beginning on line 1 (Paragraph [0258]) with the following:

BSMR protein lacking a ligand binding region inhibits ALP activity induced by Wnt3a and BMP--2. COS cells were transiently transfected with TCF--1 expression construct, ~~TOPflash~~ TOPFLASH™, pTK--Renilla, and Wnt3a. As indicated below, either BSMR, BSMRdeltaC or BSMRdeltaTM were added to the transfection mix. As a control the empty vector pcDNA3 was used for transfection. Luciferase activity was determined in cell lysates 24 hours after transfection and normalized to renilla signals. Experiments were performed in triplicate in three independent experiments. The data (plus and minus one standard deviation) for a representative experiment are presented as luciferase fold induction compared to control in FIG. 11a. In a parallel experiment C3H10T1/2 and ST2 cells were transiently transfected with Wnt3a expression vector in the presence and ~~absense~~ absence of BSMR, BSMRdeltaC and BSMRdeltaTM expression constructs. ALP activities were measured in cell lysates 72 hours after transfection and the values were normalized to protein level. The results are shown in FIG. 11b.

Please replace Figures 2, 3,5,6, and 13 with the REPLACEMENT FIGURES which are attached hereto.